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Molecularly Imprinted Polymer on Magnetic Graphene Oxide for Fast and Selective Extraction of 17β -Estradiol

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ABSTRACT: A novel nanosized substrate imprinted polymer (MIPs-GO-Fe₃O₄) was developed on a magnetic graphene oxide (GO-Fe₃O₄) surface for selective recognition and fast removal of 17 β -estradiol (17 β -E₂). The characteristics of MIPs-GO-Fe₃O₄ were evaluated by transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy. and vibrating sample magnetometer (VSM). Results suggested that GO had a thin single-layer structure anchoring Fe₃O₄ nanoparticles and that the imprinted film was coated on the GO-Fe₃O₄ surface. MIPs-GO-Fe₃O₄ was sensitive to the magnetic field and could be easily separated using an external magnet. The adsorption results indicated that the kinetic value and binding capacity of MIPs-GO-Fe₃O₄ were 0.0062 g (mg·min)⁻¹ and 4.378 μ mol g⁻¹, respectively. The Langmuir—Freundlich isotherm and pseudo-second-order kinetic models were the main adsorption mechanisms for MIPs-GO-Fe₃O₄ was also used to analyze 17 β -E₂ in real food samples, and satisfactory recoveries such as 84.20% with relative standard deviation (RSD) of 4.67% at a spike of 0.5 μ mol L⁻¹ were obtained. Thus, the MIPs-GO-Fe₃O₄-based method provided a convenient and practical platform for the separation, enrichment, and removal of 17 β -E₂ in food samples.

KEYWORDS: 17 β -estradiol, magnetic nanoparticles, graphene oxide, molecularly imprinted polymers

INTRODUCTION

17β-Estradiol (17β-E₂; E₂, 1,3,5(10)-estratriene-3,17β-diol) (Figure 1) is the most important endogenous estrogen applied in animal fattening because of its anabolic effects.¹ Some of the disadvantages of using 17β -E₂ include its toxicity and carcinogenicity; this compound may damage the endocrine system, disrupt activity in environmental water samples, and cause cancer.² To prevent uncontrolled effects on human health and deleterious effects on the environment, 17β -E₂ concentration in samples should be monitored and determined.

Nowadays, ELISA,³ HPLC,⁴ LC-MS,⁵ and GC-MS⁶ have been developed for monitoring and determining 17β -E₂ in animal tissues and the environment. Among these methods, ELISA is highly selective and sensitive and can run many analyses simultaneously.³ However, ELISA has its drawbacks, with long analysis time because of the enzyme–substrate reactions.⁷ Thus, the chromatographic techniques of HPLC, LC-MS, and GC-MS became the most frequently used methods for 17β -E₂ determination. These chromatographic methods involve traditional sample pretreatment procedures, such as solid phase extraction (SPE)⁸ and solid phase microextraction (SPME).⁹ However, the traditional SPE/SPME sorbents have drawbacks, such as low selectivity and adsorption capacity.⁸ Therefore, novel sorbents have been developed to meet the requirements of high selectivity and adsorption for 17β -E₂.

Because of high adsorption capacity, high selectivity, low cost, and ease of preparation,¹⁰ a novel type of adsorbent, namely, molecularly imprinted polymers (MIPs), has been widely applied for preconcentration and separation of trace

analytes in diverse fields, such as natural, agricultural, and food products and environmental samples.¹⁰ MIPs are prepared by copolymerization of functional monomers and cross-linkers in the presence of target analytes, which act as template molecules. After removal of the template, recognition sites that are complementary in size, shape, and functionality to the template are formed with a 3D polymer network.¹¹ The promising advantages of MIPs include desirable selectivity, physical robustness, low cost, thermal stability, and excellent reusability.¹² MIPs have become increasingly attractive in many fields, especially for SPE¹³ and chromatographic separation.¹⁴ Recently, MIPs have been developed for selective extraction and/or cleanup of 17β -E₂ from various food matrices by using a traditional bulk polymerization process.^{15,16}

As is well-known, the traditional bulk method has some drawbacks, including complicated after-treatment workup, heterogeneous binding sites, lower binding capacity, and slow mass transfer.^{15,16} Compared with the traditional bulk polymerization method, surface imprinting allows the presence of imprinted sites in the supporting material surface, ensuring the complete removal of templates, low mass-transfer resistance, and easy access to the target molecules.¹⁷ Surface imprinting over nanosized support materials with large specific surface area is appropriate for high binding capacity.¹⁸ Graphene oxide

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Figure 1. Chemical structures of 17β -estradiol, 17α -estradiol, estriol, estrone, and diethylstilbestrol.

(GO) has an extremely large specific surface area and small dimension,¹⁹ and these characteristics make GO an excellent candidate as a support material for preparing surface MIPs.²⁰ Using MIPs on GO surface (MIPs-GO) has been proposed with high loading capacity and short binding time for the template molecule.^{20,21} However, MIPs-GO homogeneously disperses in solution and is difficult to separate via traditional centrifugation and filtration methods, which restricted the application of MIPs-GO to some extent. Magnetic nanoparticles (Fe₃O₄) have properties of superparamagnetic and magnetic susceptibility, which means $\hat{F}e_3O_4$ can easily be separated from solutions by an external magnetic field.^{22,23} On the basis of the advantages of GO and Fe₃O₄, the GO-Fe₃O₄ composites have been developed and widely applied in MIPs fields.^{24,25} However, information is lacking on the development of 17β -E₂-based MIPs by using GO-Fe₃O₄ composites as the supporting material.

In the present paper, 17β -E₂-based MIPs were coated on the GO-Fe₃O₄ surface to achieve high imprinting efficiency and binding capacity. This study was the first demonstration for the preconcentration of 17β -E₂ in a food matrix using MIPs-GO-Fe₃O₄. The characteristics and binding capacity of 17β -E₂-based MIPs-GO-Fe₃O₄ were investigated in detail. The efficacy of MIPs-GO-Fe₃O₄ in extracting 17β -E₂ from food samples was also evaluated.

EXPERIMENTAL PROCEDURES

Reagents and Materials. 17 β -Estradiol (17 β -E₂), 17 α -estradiol (17 α -E₂), estrone (E₁), estriol (E₃), and diethylstilbestrol (DES) were purchased from Aladdin (Shanghai, China), and Figure 1 shows their chemical structures. Graphite powder, hexahydrate ferric chloride (FeCl₃·6H₂O), sodium acetate, and ethylene glycol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acrylic acid (AA) and 2,2'-azobis(isobutyronitrile) (AIBN) were purchased from Aladdin. Acrylamide (AM) and ethylene glycol dimethacrylate (EGDMA) were obtained from Sigma-Aldrich (Shanghai, China). Other reagents were analytically pure and used without further purification.

Synthesis of Graphene Oxide. GO was prepared using natural graphite powders according to the modified Hummers method.²⁶ Five grams of graphite powders and 2.5 g of NaNO₃ were added into a flask containing 120 mL of H_2SO_4 (95%) and cooled in an ice bath with stirring. After the graphite powders dispersed, 15 g of KMnO₄ was added gradually under stirring, and the temperature of the mixture was kept at 35 °C for 60 min. H_2O_2 (3 mL, 30%) and deionized water (100 mL) were added slowly into the above reaction mixture at 90 °C with vigorous agitation for 30 min. Finally, the resultant yellow-brown

GO was washed with a 10% HCl aqueous solution and distilled water until the pH was 7.0 and then was dried at 40 $^\circ C$ for 24 h.

Synthesis of Magnetic Graphene Oxide (GO-Fe₃O₄). The GO-Fe₃O₄ nanocomposites were synthesized according to the solvothermal method as follows:²⁷ 0.45 g of GO was completely dispersed in 70 mL of ethylene glycol via ultrasound for more than 3 h; then 1.4 g of FeCl₃·6H₂O and 2.8 g of NaOAc were added into the above mixture. After stirring for about 30 min, the mixture was transferred into a 100 mL Teflon stainless steel autoclave and reacted at 200 °C for 8 h. The obtained GO-Fe₃O₄ nanocomposites were thoroughly washed with distilled water and ethanol and then freeze-dried.

Modification of GO-Fe₃**O**₄ **Nanocomposites.** The surface of GO-Fe₃O₄ nanocomposites was grafted with AA by the following procedures:²⁸ Briefly, 5 mL of the suspension containing 100 mg of GO-Fe₃O₄ nanocomposites was added into 15 mL of ethanol with ultrasonication, and 1 mL of AA was then added into the above mixture with shaking at a rate of 300 rpm for 2 h.

Preparation of MIPs-GO-Fe₃O₄. The 17β -E₂-based MIPs-GO-Fe₃O₄ was prepared using AM as functional monomer, EGDMA as cross-linker, and AIBN as initiator in acetonitrile containing surface supporting materials of GO-Fe₃O₄-AA. 17β -E₂ (0.272 g, 1 mmoL), AM (0.517 g, 6 mmoL), EGDMA (170 µL, 0.90 mmoL), and AIBN (0.062 g, 0.381 mmoL) were added into 60 mL of acetonitrile containing GO-Fe₃O₄-AA (80 mg) in a 250 mL thick-walled glass tube. The prepolymerization mixture was degassed by using an ultrasonic bath for 10 min, then purged with nitrogen for 15 min to remove oxygen, and polymerized at 60 °C for 24 h. The MIPs-GO-Fe₃O₄ particles were washed with methanol/acetic acid solution (9:1, v/v) to remove completely the template molecules and residual monomers. Finally, the particles were dried to constant weight under vacuum at 55 °C. As control, non-imprinted polymers (NIPs-GO- Fe_3O_4) were synthesized simultaneously under identical conditions but omitting the template in the reaction system.

Characterization. Fourier transform infrared (FT-IR) spectra were recorded by using a FT-IR spectrophotometer (Nicolet 5700, Thermo Electron Corp., Waltham, MA, USA). The X-ray diffractometer (XRD) patterns were recorded using an XRD analyzer (D8-FOCUS, Bruker, Karlsruhe, Germany). Transmission electron microscopy (TEM) images were obtained on a JEOL (JEM-2010HR, Japan) transmission electron microscope. The magnetic properties were examined by vibrating specimen magnetometer (VSM) (7407; Lake Shore Cryotronics, Westerville, OH, USA). Raman spectra were collected using INVIA spectrophotometer (Renishaw, UK).

Adsorption Experiments. To investigate the binding capacity of MIPs-GO-Fe₃O₄ particles, a static adsorption test was conducted in acetonitrile solutions as follows:¹⁵ 20 mg of MIPs-GO-Fe₃O₄ particles was dispersed in a 10 mL flask containing 2.0 mL of 17β -E₂ solutions of various concentrations within 0.1–1.0 mmol L⁻¹, respectively. After shaking for 12 h at room temperature, the samples were separated by

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Figure 2. Schematic illustration of the preparation processes of MIPs-GO-Fe₃O₄.



Figure 3. TEM images of GO (A), GO-Fe₃O₄ (B), and MIPs-GO-Fe₃O₄ (C).

an external magnet. The free concentration of 17β - E_2 in the supernatant solutions was determined using a UV spectrometer at 280.0 nm (TU-1810; Beijing Purkinje General Instrument Co., Ltd.). The adsorption amount of 17β - E_2 on MIPs-GO-Fe₃O₄ particles (*Q*) was calculated according to the following equation; *Q* tests were performed in triplicate with data reported as the mean values:

$$Q = (C_0 - C_F)V/M$$

 $C_0 \text{ (mg mL}^{-1})$ and $C_F \text{ (mg mL}^{-1})$ are the initial and final 17β - E_2 solution concentrations, respectively. V (mL) is the sample volume, and M (g) is the mass of MIPs-GO-Fe₃O₄ particles.

Dynamic adsorption experiments were also performed. MIPs-GO-Fe₃O₄ (20 mg) or NIPs-GO-Fe₃O₄ (20 mg) were dispersed into 2.0 mL acetonitrile solutions containing 17β -E₂ (0.5 mmol L⁻¹), respectively. The solution was separated, and the free concentration of 17β -E₂ in the supernatant solutions was determined using UV spectrometry at 280.0 nm after 5, 10, 15, 20, 25, 30, 35, and 40 min of stirring, respectively.

Selectivity experiments were conducted using 17α -E₂, E₁, E₃, and DES as structural analogues. MIPs-GO-Fe₃O₄ (20 mg) or NIPs-GO-Fe₃O₄ (20 mg) were dispersed into 2.0 mL acetonitrile solutions containing 0.5 mmol L⁻¹ of 17β -E₂, 17α -E₂, E₁, E₃, and DES, respectively. The amount of 17β -E₂, 17α -E₂, E₁, E₃, and DES in the supernatant was determined by UV after shaking for 12 h at room temperature. The recognition ability of MIPs-GO-Fe₃O₄ was evaluated by imprinting factor (α), which is defined in the equation

 $\alpha = Q_{\rm MIP}/Q_{\rm NIP}$

where Q_{MIP} and Q_{NIP} are the adsorption amounts of the template or analogues on MIPs-GO-Fe₃O₄ and NIPs-GO-Fe₃O₄, respectively.

Analysis of 17β -E₂ in Real Samples. Milk powder samples were purchased from a local market. The milk powder samples (2 g)

without spiking or spiked with 17β -E₂ standard solutions (2 mL; 0.2, 0.5, or 1.0 μ mol L⁻¹) were first extracted using acetonitrile (20 mL) for 30 min. Then the solutions were centrifuged and filtered, and the extract solutions were obtained. MIPs-GO-Fe₃O₄ particles (20 mg) were added to the extract solution. The solution was shaken at room temperature for 1 h. A magnet (N₃₅ model) was used to separate MIPs-GO-Fe₃O₄ particles from the solution, and the supernatant was measured by UV to determine the amounts of 17β -E₂. Simultaneously, the 17β -E₂ adsorbed onto MIPs-GO-Fe₃O₄ sorbent was subsequently eluted with 2 mL of methanol/acetic acid (9:1, v/v). After desorption for 2 h, the template molecule 17β -E₂ and adsorbent were easily rapidly separated by the magnet. The solution containing template molecule 17β -E₂ was dissolved in 2 mL of acetonitrile solvent, and the recovery amount of 17β -E₂ was determined by UV at 280.0 nm. All tests were performed in triplicate with data reported as the mean values. It should be noted that matrix interferences could be excluded by using the selective MIPs-GO-Fe₃O₄ as extracting agent. Moreover, the absorbance values were measured by deducting the blank values.

RESULTS AND DISCUSSION

Fabrication of MIPs-GO-Fe₃O₄. Figure 2 illustrates the synthesis route of MIPs-GO-Fe₃O₄. GO was prepared according to the classic Hummers method using graphite powders as starting material. GO-Fe₃O₄ nanocomposites were fabricated by a solvothermal method, GO was dissolved in ethylene glycol solution, and FeCl₃·6H₂O and NaOAC were used as the iron source and reductant, respectively. The Fe³⁺ could be absorbed by oxygen-containing functional groups of GO, and the Fe³⁺ was reduced to Fe₃O₄ by NaOAC during the reaction. GO-Fe₃O₄ nanocomposites were functionalized by a simple coordination reaction of the carboxyl group of AA with

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Figure 4. FT-IR spectra (A) of GO, GO-Fe₃O₄, and MIPs-GO-Fe₃O₄ (1, GO; 2, GO-Fe₃O₄; 3, MIPs-GO-Fe₃O₄) and VSM (B) (1, Fe₃O₄; 2, GO-Fe₃O₄; 3, MIPs-GO-Fe₃O₄).

the oxygen-containing functional groups of GO and particle surface residual Fe³⁺. AA monomer grafted to GO-Fe₃O₄ surfaces and formed GO-Fe₃O₄-AA with vinyl end groups.^{29,30} The 17 β -E₂-based MIPs-GO-Fe₃O₄ was developed at 60 °C for 24 h in the presence of nanosized supporting materials (GO-Fe₃O₄-AA), template molecule (17 β -E₂), functional monomer (AM), and cross-linking agent (EGDMA). The imprinted monolayer will polymerize at the GO-Fe₃O₄ surface by the reaction between AA and AM.^{31,32} Finally, MIPs-GO-Fe₃O₄ was fabricated after washing with methanol/acetic acid (9:1, v/ v) to remove the template molecule of 17 β -E₂.

Characterization of MIPs-GO-Fe₃O₄. The prepared samples were characterized by TEM, FT-IR, Raman, XRD, and VSM. Figure 3 shows the morphologies of GO, GO-Fe₃O₄, and MIPs-GO-Fe₃O₄, respectively. GO displays a thin and irregular paper-like morphology with some wrinkles (Figure 3A). Fe₃O₄ nanoparticles were formed and homogeneously anchored onto the GO sheet (Figure 3B). As shown in Figure 3C, the MIPs were firmly attached on the surface of GO-Fe₃O₄ after polymerization.

Figure 4A shows the FT-IR spectra results of GO, GO- Fe_3O_4 , and MIPs-GO- Fe_3O_4 . The three samples have the same adsorption peak at 3419.4 cm⁻¹ because of the O–H stretching vibration. For the FT-IR spectra of GO, the peak at 1251.3



Figure 5. Raman (A) (1, GO; 2, GO-Fe₃O₄; 3, MIPs-GO-Fe₃O₄) and XRD (1, GO-Fe₃O₄; 2, MIPs-GO-Fe₃O₄; 3, GO).

cm⁻¹ characterized C–O stretching vibration of the epoxy groups. A strong Fe–O characteristic stretching vibration peak of 544.0 cm⁻¹ appeared for GO-Fe₃O₄, which indicated that Fe₃O₄ nanoparticles were successfully anchored onto the GO sheets. For the FT-IR spectra of MIPs-GO-Fe₃O₄, the absorption peaks of 2366.2 and 1585.6 cm⁻¹ were attributed to C=O stretching vibration of AA monomer.²⁸ The intensity of C=O stretching (1728.0 cm⁻¹) was remarkably increased when Fe₃O₄ particles were covered with MIPs containing a large number of C=O groups in polyacrylamide units, which indicated the formation of imprinted polymers on the GO-Fe₃O₄ surface.

Figure 4B shows the magnetic property of Fe_3O_4 , $GO-Fe_3O_4$, and MIPs-GO-Fe₃O₄, and the inset illustrates the dispersion and agglomeration processes of MIPs-GO-Fe₃O₄. The remanence and coercivity of samples almost disappeared, indicating that Fe_3O_4 , $GO-Fe_3O_4$, and MIPs-GO-Fe₃O₄ have superparamagnetic characteristics. The saturation magnetization of Fe_3O_4 , $GO-Fe_3O_4$, and MIPs-GO-Fe₃O₄ gradually decreased 41.14, 35.37, and 11.11 emu g⁻¹, respectively. Saturation magnetization decreased for MIPs-GO-Fe₃O₄ because of the presence of the GO and MIPs polymers on the Fe_3O_4 surface. Fortunately, the saturation magnetization value of MIPs-GO-Fe₃O₄ (11.11 emu g⁻¹) was sufficient to ensure the easy and quick separation of the compounds from solutions. As shown in the inset of Figure 4B, the MIPs-GO-



Figure 6. Static adsorption isotherms (A) and kinetic binding curves (B) of 17β -E₂ onto MIPs-GO-Fe₃O₄ (1) and NIPs-GO-Fe₃O₄ (2). Data are represented as the mean \pm standard deviation (SD) (*n* = 3).

 $\rm Fe_3O_4$ can homogeneously disperse in aqueous solutions and is also sensitive to the magnetic field, showing MIPs-GO-Fe_3O_4 can be separated easily by the external magnet.

Figure 5A shows the Raman spectra of GO, GO-Fe₃O₄, and MIPs-GO-Fe₃O₄. All Raman spectra showed that three samples

had G (approximately 1580 cm⁻¹) and D bands (approximately 1340 cm⁻¹), which agreed with the usual features of carbon materials in the Raman spectra. The valued of I_D/I_G wrtr approximately 0.923, 1.24, and 1.38 for GO, GO-Fe₃O₄, and MIPs-GO-Fe₃O₄, respectively.³³ The average size of the inplane graphitic crystallite sp² domains increased, and such increase may be due to the small partial reduction of GO during synthesis.

The corresponding (XRD) patterns of GO, GO-Fe₃O₄, and MIPs-GO-Fe₃O₄ are presented in Figure 5B. GO showed a very sharp diffraction peak at 2θ = 7.40 corresponding to a *d*-spacing of 12.45 nm. However, the sharp peak of 2θ = 7.40 decreased for GO-Fe₃O₄ and MIPs-GO-Fe₃O₄, showing that GO was reduced to some extent by NaOAC during preparation. Typical XRD pattern peaks of Fe₃O₄ were observed at 2θ values of 30.0, 35.7, 42.9, 53.6, 56.8, and 62.7 for GO-Fe₃O₄ and MIPs-GO-Fe₃O₄ and MIPs-GO-Fe₃O₄ and MIPs-GO-Fe₃O₄ and MIPs-GO-Fe₃O₄ and MIPs-GO-Fe₃O₄ and MIPs-GO-Fe₃O₄ and the standard XRD data of Fe₃O₄ (JCPDS Card 019-0629). The XRD pattern results showed that MIPs-GO-Fe₃O₄ comprised Fe₃O₄ nanoparticles, and the synthesized processes did not change the XRD phase of Fe₃O₄.

Binding Studies of MIPs-GO-Fe₃O₄. The static, dynamic, and selective binding abilities of MIPs-GO-Fe₃O₄ were investigated. Figure 6A shows the static binding isotherms of 17β -E₂ on MIPs-GO-Fe₃O₄. The adsorption capacity of MIPs-GO-Fe₃O₄ for 17β -E₂ increased quickly with increasing concentration. When the equilibrium concentration reached 0.9 mmol L^{-1} , the adsorption amounts of MIPs-GO-Fe₃O₄ appeared stable, and such stability may be due to the recognition sites, which were almost completely occupied by 17β -E₂. Figure 6A also shows that the NIPs-GO-Fe₃O₄ had lower saturated adsorption amounts than MIPs-GO-Fe₃O₄ because of the nonspecific recognition sites in NIPs-GO- Fe_3O_4 . Moreover, the equilibrium dissociation constant (k_d) and maximum adsorption capacity (q_{max}) of the Scatchard equation were calculated, as follows: 12.68 μ g mL⁻¹ and 5.44 μ mol g⁻¹ for MIPs-GO-Fe₃O₄ and 7.65 μ g mL⁻¹ and 1.42 μ mol g^{-1} for NIPs-GO-Fe₃O₄, respectively. The obtained q_{max} well

Table 1. Parameters Obtained of 17β -E₂ Adsorption onto MIPs-GO-Fe₃O₄ and NIPs-GO-Fe₃O₄ from Four Adsorption Isotherm Models

isotherm model	equation	parameter	MIPs-GO-Fe ₃ O ₄	NIPs-GO-Fe ₃ O ₄
Langmuir	C_{e} 1 C_{e} 1	R^{2a}	0.9143	0.9276
-	$\frac{1}{Q_e} = \frac{1}{Q_e} C_e + \frac{1}{k_I Q_{e}}$	Q_{\max}^{b}	2.154 ± 0.543	0.965 ± 0.038
		$k_{ m L}{}^c$	0.021 ± 0.003	0.034 ± 0.003
Freundlich	1	R^2	0.9430	0.9287
	$\ln Q_{\rm e} = -\frac{1}{n} \ln C_{\rm e} + \ln k_{\rm F}$	$k_{\rm F}^{d}$	0.168 ± 0.028	0.069 ± 0.009
		$1/n^e$	0.689 ± 0.042	0.586 ± 0.037
Scatchard	0 (0 - 0)	R^2	0.9172	0.9236
	$\frac{c_e}{C} = \frac{c_{max}}{k}$	$Q_{\rm max}$	5.437 ± 0.489	1.415 ± 0.049
	$C_e \kappa_d$	$k_{\rm d}^{f}$	12.68 ± 12. 41	7.65 ± 0.98
Langmuir—Freundlich	$(1, 1, (1))^m$	R^2	0.9872	0.9743
	$\frac{1}{B} = \frac{1}{N\alpha} \left(\frac{1}{E} \right) + \frac{1}{N}$	N. ^g	59.28 + 14.31	6.365 + 1.128
	$D = V_t u (1) = V_t$	α^h	0.038 ± 0.003	0.034 ± 0.001
		m^i	0.682 + 0.034	0.825 + 0.028

^{*a*}Correlation coefficient. ^{*b*}Maximum absorption capacity. ^{*c*}Langmuir constant. ^{*d*}Freundlich constant. ^{*e*}Related to adsorption intensity and surface heterogeneity. ^{*f*}Scatchard constant. ^{*g*}Total number of binding sites. ^{*h*}Related to the median binding affinity constant K_0 via $K_0 = \alpha_1/m$. ^{*i*}Heterogeneity index, ranging from 0 to 1,equal to 1 for homogeneous materials.

kinetic model	equation	parameters	MIPs-GO-Fe ₃ O ₄	NIPs-GO-Fe ₃ O ₄
pseudo-first-order	$\ln(Q_{e} - Q_{t}) = \ln Q_{e} - k_{1}t$	R^{2a} k_1^{b} Q_e^{c}	$\begin{array}{l} 0.9675 \\ 0.034 \pm 0.003 \\ 1.926 \pm 0.325 \end{array}$	$\begin{array}{c} 0.9376 \\ 0.042 \pm 0.004 \\ 0.872 \pm 0.024 \end{array}$
pseudo-second-order	$\frac{t}{Q_{t}} = \frac{1}{k_2 Q_e^2} + \frac{t}{Q_e}$	$R^2 \ k_2{}^d \ Q_e$	0.9912 0.0062 ± 0.0023 4.378 ± 0.154	0.9864 0.0473 ± 0.0028 0.952 ± 0.024

Table 2. Parameters Obtained of 17β -E₂ Adsorption toward MIPs-GO-Fe₃O₄ and NIPs-GO-Fe₃O₄ from Two Kinetic Adsorption Models

^{*a*}Correlation coefficient. ^{*b*}Rate constant of adsorption in pseudo-first-order model. ^{*c*}Saturated adsorption amount of template molecule at equilibrium. ^{*d*}Rate constant of adsorption in pseudo-second-order model.



Figure 7. Competitive binding of 17β -E₂, 17α -E₂, E₃, E₁, and DES on MIPs-GO-Fe₃O₄ and NIPs-GO-Fe₃O₄ (A), and recoveries (%) and RSD (%) of MIPs-GO-Fe₃O₄ obtained from analysis of milk powder samples spiked with different concentrations (0.2, 0.5, and 1.0 mmol L⁻¹) 17β -E₂ (B). Data are represented as the mean \pm SD (n = 3).

agreed with the experimental result (3.95 μ mol g⁻¹). The results suggested that the MIPs-GO-Fe₃O₄ had excellent binding capacity for the template molecule.

Moreover, the adsorption process was further studied by four classical isotherm models including Langmuir, Freundlich, Scatchard, and Langmuir—Freundlich.³⁴ The corresponding equations and parameters of these models for adsorption of 17β -E₂ onto the MIPs-GO-Fe₃O₄ and NIPs-GO-Fe₃O₄ are listed in Table 1. The best fit was obtained from the Langmuir—

Freundlich model, which resulted in a correlation coefficient of 0.9872. The parameters N_{tr} α , and *m* were 59.28, 0.038, and 0.682 for MIPs-GO-Fe₃O₄ and 6.365, 0.034, and 0.825 for NIPs-GO-Fe₃O₄, respectively. An excellent imprinting effect was observed from the presence of a number of specific binding sites on the MIPs-GO-Fe₃O₄ surface.

Dynamic binding experiments were performed to investigate the mass transfer properties of MIPs-GO-Fe₃O₄, and the results are shown in Figure 6B. The adsorption amounts of MIPs-GO- Fe_3O_4 for 17β -E₂ increased and reached equilibrium within 10 min. The rapid adsorption and high equilibrium binding capacity (381.2 μ g g⁻¹) were due to the high ratio of surfaceimprinted sites and large surface-to-volume ratio of MIPs-GO-Fe₃O₄, which allow high binding capacity and fast mass transfer. To investigate the mechanism underlying the biosorption process, such as mass transfer and chemical reaction, the pseudo-first-order and pseudo-second-order kinetic models were used to test the experimental data of MIPs-GO-Fe₃O₄ and NIPs-GO-Fe₃O₄.³⁵ The results are shown in Table 2. The pseudo-second-order model could better fit the time effect on the adsorption system than other kinetic models. The pseudosecond-order model provided the most suitable correlation for the adsorption, with the highest correlation coefficient of 0.9912. The obtained rate constant (k_2) was 0.0062 g (mg· min)⁻¹, and the equilibrium adsorption capacity (Q_e) was 4.378 μ mol g⁻¹. Therefore, the adsorption could be deduced to follow the pseudo-second-order kinetic model.

To investigate the binding specificity of the MIPs-GO-Fe₃O₄, a selectivity test was conducted using structural analogues of 17β -E₂, being 17α -E₂, E₃, E₁, and DES, as control compounds. As shown in Figure 7A, the adsorption capacity of NIPs-GO-Fe₃O₄ is very close and nonselective for the five compounds because selective recognition sites are absent in NIPs-GO-Fe₃O₄. However, MIPs-GO-Fe₃O₄ showed a significantly higher binding capacity for 17β -E₂ than for these competitive analogues. The binding capacities of MIPs-GO-Fe₃O₄ for 17β - E_2 , 17 α - E_2 , E_3 , E_1 , and DES were 640, 425, 376, 278, and 216 $\mu g g^{-1}$, respectively. Accordingly, the imprinting factor values (α) of MIPs-GO-Fe₃O₄ for 17 β -E₂, 17 α -E₂, E₃, E₁, and DES were 2.46, 1.80, 1.64, 1.23, and 1.11, respectively. These results indicated that MIPs-GO-Fe₃O₄ has highly specific recognition ability for 17β -E₂. The high selectivity for 17β -E₂ may be based on two factors. The first factor is the difference in chemical structures between 17β -E₂, 17α -E₂, E₃, E₁, and DES. The second factor is that only 17β -E₂ can specifically match the binding sites with the template in terms of size and shape.

Method Validation and Application to Analysis of 17β -E₂ in Food Samples. To further assess the practical applicability of MIPs-GO-Fe₃O₄, milk powder samples were

used as real sample in the analysis. Figure 7B shows the extraction recoveries of MIPs-GO-Fe₃O₄ at three spiked 17β -E₂ concentrations of 0.2, 0.5, and 1.0 μ mol L⁻¹, that is, 72.63, 84.20, and 68.93% with relative standard deviations (RSDs) of 5.26, 4.67, and 4.92%, respectively. The limits of detection and quantification were determined to be 0.035 and 0.10 μ mol L⁻¹ on the basis of signal-to-noise ratios of 3 and 10, respectively, and the regression equation was attained, y = 0.0933x + 0.2152 $(R^2 = 0.9928)$ within 0.1–1.0 mmol L⁻¹. It is noted that the separation process was finished quickly within 10 s when a magnet was used. Compared to conventional MIPs extraction, a simpler, faster, and more economical magnetic separation procedure was provided, dispensing with pretreatment such as filtration or centrifugation. Thus, MIPs-GO-Fe₃O₄ can be used for preconcentration, separation, extraction, and removal of 17β -E₂ in real food samples.

In conclusion, a new type of molecularly imprinted nanomaterial (MIPs-GO-Fe₃O₄) was developed for the specific recognition and highly effective removal of 17β -E₂ in complicated matrices. MIPs-GO-Fe₃O₄ was prepared using GO-Fe₃O₄, AM, and 17β -E₂ as the supporting material, monomer, and template molecule, respectively. The magnetic property of MIPs-GO-Fe₃O₄ allowed the simple, rapid, and efficient separation of 17β -E₂ from matrices. Owing to the extremely large area of GO-Fe₃O₄, MIPs-GO-Fe₃O₄ has faster desorption and adsorption dynamics and higher selectivity than other traditional MIPs. MIPs-GO-Fe₃O₄ also showed excellent selectivity toward template molecules. In addition, MIPs-GO- Fe_3O_4 displayed satisfactory 17β -E₂ recovery when used for enrichment and removal of 17β -E₂ from food samples. We have provided an excellent platform for pretreatment and removal of 17β -E₂ from contaminated environmental and food samples.

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Notes

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